Regulation of human Δ-6 desaturase gene transcription: identification of a functional direct repeat-1 element

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Abstract **The rate-limiting step in 20:4(n-6) and 22:6(n-3) synthesis is the desaturation of 18:2(n-6) and 18:3(n-3) by Δ-6 desaturase. In this report, we demonstrate that n-6 and n-3 PUFAs suppressed the hepatic expression of rodent Δ-6 desaturase by inhibiting the rate of Δ-6 desaturase gene transcription. In contrast, consumption of the peroxisome proliferator-activated receptor (PPAR)α activator WY 14,643 significantly enhanced the transcription of hepatic Δ-6 desaturase by more than 500%. Transfection reporter assays with HepG2 cells revealed that the PUFA response region for the human Δ-6 desaturase gene involved the proximal promoter region of −283/+1 human Δ-6 desaturase gene, while the WY 14,643 response element (RE) was identified as an imperfect direct repeat (DR-1) located at −385/−373. The WY 14,643 induction of the human Δ-6 desaturase promoter activity was dependent upon the expression of PPARα. Electrophoretic mobility shift assays revealed that nuclear proteins extracted from HepG2 cells expressing PPARα specifically interacted with the −385/−373 DR-1 sequence of the human Δ-6 desaturase gene. The interaction was eliminated by the unlabeled PPARα RE of the rat acyl-CoA oxidase gene, and the protein-DNA complex was super-shifted by treatment with anti-PPARα. The −385/−373 sequence also interacted with a mixture of in vitro translated PPARα-retinoic acid receptor X (RXR)α, but by themselves neither PPARα nor RXRα could bind to the Δ-6 desaturase DR-1. These data indicate that the 5′-flanking region of the human Δ-6 desaturase gene contains a DR-1 that functions in the regulation of human Δ-6 desaturase gene transcription, and thereby plays a role in the synthesis of 20- and 22-carbon polyenoic fatty acids.**—Tang, C., H. P. Cho, M. T. Nakamura, and S. D. Clarke. **Regulation of human Δ-6 desaturase gene transcription: identification of a functional direct repeat-1 element. *J. Lipid Res.* 2003.** 44: **686–695.**

Supplementary key words liver • peroxisome proliferator-activated receptor α • polyunsaturated fatty acids

Long chain PUFAs such as 20:4(n-6) and 22:6(n-3) play pivotal roles in a number of biological functions, including brain development, cognition, inflammatory responses, and hemostasis (1–4). Over 30% of the fatty acid in brain phospholipid consists of 20:4(n-6) and 22:6(n-3), and ~50% of the fatty acid in the retina is 22:6(n-3) (5, 6). An inadequate availability of 20:4(n-6) such as occurs in diabetes is associated with impaired nerve transmission (7, 8). In addition to being vital components of membrane phospholipids and functioning in key steps of cell signaling, 20- and 22-carbon PUFAs govern the expression of a wide array of genes. In particular, they down-regulate the expression of hepatic lipogenic genes while they up-regulate the genes encoding proteins of fatty acid oxidation (9–13).

Some of the daily needs for 20- and 22-carbon n-6 and n-3 PUFA are fulfilled from dietary constituents (e.g., meat and fish). However, most of the 20- and 22-carbon PUFAs found in human tissues are derived from the desaturation and elongation of 18:2(n-6) and 18:3(n-3), and the conversion of these precursor fatty acids to their respective 20- and 22-carbon polyenoic fatty acid products. The rate of flux of 18:2(n-6) and 18:3(n-3) to their respective 20- and 22-carbon polyenoic fatty acid products is determined by the activity of Δ-6 desaturase and Δ-5 desaturase (14–16). Δ-6 Desaturase and Δ-5 desaturase are microsomal enzymes that are expressed in nearly all human tissues, with the greatest activities found in the liver, heart, and brain (14–16). Traditionally, Δ-6 desaturase and Δ-5 desaturase were thought to be components of a three-enzyme system that involves NADH-cytochrome b_5 reductase and cytochrome b_5 (14). However, the open reading frame sequence for Δ-6 desaturase and Δ-5 desaturase suggests that, unlike stearoyl-CoA desaturase, the human Δ-6 desaturase and Δ-5 desaturase enzymes contain a cytochrome b_5 domain as part of the peptide itself (15, 16). This cytochrome b_5 domain is also present in the

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Abbreviations: EMSA, electrophoretic mobility shift assay; HNF-4, hepatic nuclear factor 4; nt, nucleotide; PPAR, peroxisome proliferator-activated receptor; RLU, relative light unit; RXR, retinoic acid receptor X; SREBP-1, sterol regulatory element binding protein-1.

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TABLE 1. Effects of dietary PUFAs and WY 14,643 on rat liver Δ-6 desaturase gene transcription

Hepatic Gene Transcript	Transcription Activity				
	WY	FO	SO	TO	FF
Δ-6 desaturase	27.5 ± 6.1[a]	ND	1.8 ± 0.7[a]	4.0 ± 1.1	4.5 ± 1.2
FAS	55.2 ± 24.0[a]	18.5 ± 4.2[a]	43.0 ± 9.2[a]	135 ± 28.0	127 ± 15.0
AOX	67.5 ± 7.9[a]	5.0 ± 2.0	6.8 ± 2.0	4.0 ± 1.0	4.3 ± 1.5

The hepatic transcription of Δ-6 desaturase, fatty acid synthase (FAS), and acyl-CoA oxidase (AOX) genes in rats fed a fat-free (FF) diet or the FF diet supplemented with 0.1% WY 14,643 (WY), 10% (w/w) fish oil (FO), safflower oil (SO), or triolein oil (TO) was determined by nuclear run-on assay. Transcription activities (dpm/transcript per 10^6 dpm total RNA) were corrected for nonspecific hybridization to the pBS vector, and data are expressed as means ± SE; n = 4 rats/treatment. ND, nondetectable.

[a] Indicates a significant difference from the FF values ($P < 0.05$).

rat Δ-6 desaturase and the mouse Δ-5 desaturase (15–18). In addition to the b_5 domain, the Δ-6 desaturase and Δ-5 desaturase proteins possess several other common features, including an identical peptide size, two membrane-spanning regions, and three histidine-rich regions (15–18). Moreover, the activity of both enzymes changes in concert with alterations in nutritional and hormonal status (15, 16, 19–22). For example, dietary n-6 and n-3 PUFA significantly lower the hepatic abundance of Δ-6 desaturase and Δ-5 desaturase mRNA, and this is paralleled by a comparable reduction in enzyme activity (15, 16). On the other hand, ingestion of peroxisome proliferator-activated receptor (PPAR)α activators (e.g., fibrates) significantly increases the hepatic abundance of Δ-6 desaturase mRNA and enzymatic activity (19, 21). This rise in Δ-6 desaturase and Δ-5 desaturase enzymatic activity is paralleled by greater hepatic production of 20- and 22-carbon n-6 and n-3 polyenoic fatty acids (21), and by an enrichment of peripheral tissues with 20- and 22-carbon n-6 and n-3 polyenoic fatty acids (H. P. Cho and S. D. Clarke, unpublished observations).

The mechanisms by which dietary PUFA and PPARα activators regulate hepatic Δ-6 desaturase and Δ-5 desaturase gene expression are unknown. In this report, we demonstrate that PUFAs suppress and non-PUFA PPARα ligand activators induce transcription for both the rat and human liver Δ-6 desaturase gene. Moreover, we have determined that the human Δ-6 desaturase gene contains an imperfect direct repeat-1 (DR-1) at −385/−373 that imparts PPARα responsiveness to the Δ-6 desaturase promoter.

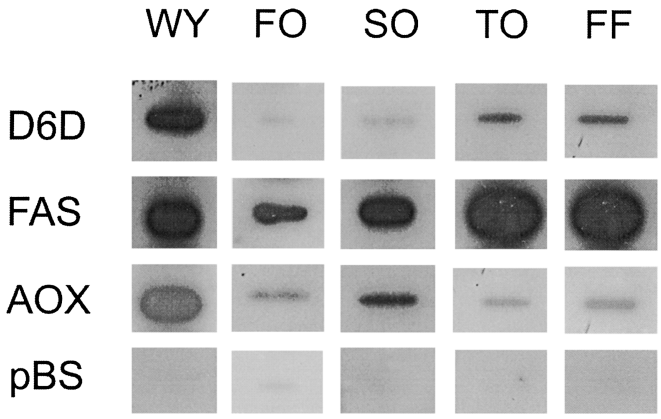

Fig. 1. Transcriptional regulation of rat liver Δ-6 desaturase by WY 14,643 (WY) and PUFA. Nuclear run-on assays were conducted using nuclei isolated from rats fed the fat-free (FF) diet or the FF diet supplemented with 0.1% WY, 10% fish oil (FO), safflower oil (SO), or triolein (TO). FAS, fatty acid synthase; AOX, acyl-CoA oxidase. A quantitative summary of the nuclear run-on output is presented in Table 1.

MATERIALS AND METHODS

Dietary study

Male Sprague Dawley rats (Harlan Sprague-Dawley) were housed in a temperature- and light-controlled environment and

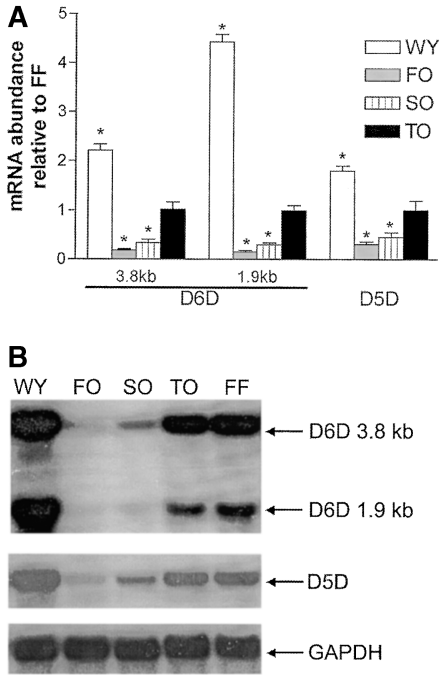

Fig. 2. The effects of WY and PUFA on rat liver Δ-6 desaturase and Δ-5 desaturase mRNA abundance. A: Hepatic Δ-6 desaturase and Δ-5 desaturase mRNA abundance was quantified in rats fed a FF diet or the FF diet supplemented with 0.1% WY or 10% (w/w) FO, SO, or TO. Data are expressed as means ± SE, n = 4. Asterisk indicates that the values are significantly different from the FF diet group ($P < 0.05$). B: A representative Northern blot (30 μg/lane) showing the 3.8 kb and 1.9 kb transcripts for Δ-6 desaturase (D6D) plus the Δ-5 desaturase (D5D) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts.

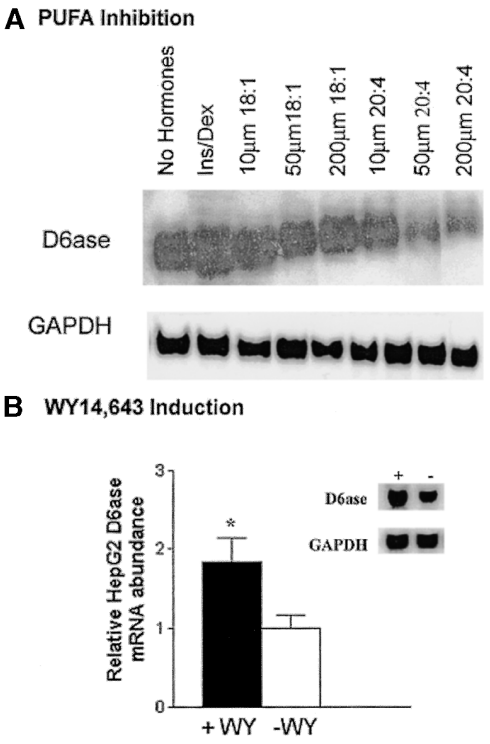

Fig. 3. WY induction of Δ-6 desaturase expression in HepG2. A: A representative Northern blot (30 μg total RNA/lane) depicting the effect of 18:1(n-9) and 20:4(n-6) on the HepG2 abundance of Δ-6 desaturase and GAPDH mRNA. Confluent HepG2 cells maintained for 48 h in a serum-free medium that contained 10^{-7} M insulin (Ins) and dexamethasone (Dex) were treated with varying concentrations of albumin-bound 18:1(n-9) or 20:4(n-6) (11). B: HepG2 cells were transfected with pSG5.m peroxisome proliferator-activated receptor (PPAR)α and incubated for 36 h with (+) and without (−) the PPARα-specific activator, WY. Δ-6 Desaturase and GAPDH mRNA abundance was quantified by Northern analysis (30 μg/lane). The bar graph depicts relative Δ-6 desaturase mRNA abundance ± SE (n = 3), and the inset represents a pooled Northern blot for the triplicate samples. Asterisk denotes that WY significantly increased relative abundance of Δ-6 desaturase mRNA, $P < 0.05$.

adapted to a 3 h per day (9–12) meal-feeding regimen using a high-glucose, fat-free diet (Dyets, Bethlehem, PA) (23). After a 7-day adaptation period, the rats were randomly assigned to one of the following dietary treatments (n = 5 rats per group) and fed for an additional 5 days: a high glucose, fat-free diet; the fat-free diet plus 0.1% (w/w) WY 14,643 (Chemsyn Science Labs, Lenexa, KS); or the fat-free diet supplemented with 10% fat (w/w) as triolein [99% 18:1(n-9)], safflower oil [65% 18:2(n-6)], or sterol-free, menhaden fish oil [35% 20:5 and 22:6(n-3)]. Nuclei for nuclear run-on assays, and RNA for measures of transcript abundance were isolated from the rats immediately after the last 3 h meal (see below).

RNA analysis and gene transcription

The abundance of a variety of hepatic transcripts described in the figures was determined by Northern blot analysis using total RNA extracted by the phenol-guanidinium isothiocyanate method (24). The abundance of specific transcripts of interest were quantified following hybridization with cDNA probes labeled with [α-^{32}P]dCTP (Amersham, Arlington Heights, IL) using polymerase chain reaction radiolabeling or random prime labeling (Life Technologies, Baltimore, MD) (11, 16). The impact of WY 14,643 and various dietary fats on the in vivo transcription of rat liver Δ-6 desaturase, fatty acid synthase, and acyl-CoA oxidase (AOX) was determined using the nuclear run-on assay procedure (11, 25). Equivalent counts of nuclear RNA labeled with [α-^{32}P]UTP (Amersham) were hybridized for 72 h at 40°C to filter-bound cDNAs specific for Δ-6 desaturase, FAS, and AOX. After hybridization and washing, the membranes were exposed to X-ray film (X-OMAT-AR, Kodak, Rochester, NY). Each RNA hybrid was cut out and counted by liquid scintillation counting. Transcription and mRNA abundance data were subjected to one-way ANOVA, and treatment effects ($P < 0.05$) were determined as differences from the fat-free group.

Genomic cloning and reporter vector construction for human Δ-6 desaturase

Human Δ-6 desaturase and Δ-5 desaturase cDNA sequences were used to BLAST search the human genomic database. Clone PAC AC004228 corresponding to the region of human chromosome 11q12.2–13.1 was found to contain all of the exons for Δ-6 desaturase and Δ-5 desaturase, as well as the entire region spanning the distance between the two genes. A *Kpn*I-*Avi*II fragment representing the sequence of −6,249 to +279 was cut from the human clone AC004228. A luciferase (LUC) reporter construct containing the Δ-6 desaturase proximal promoter region of −118/+132 was prepared by cutting the −6,249/+279 fragment with *Sac*I and *Nae*I, and subsequently inserting the −118/+132 sequence into the *Sac*I and *Sma*I sites of pGL3.LUC basic vector (Promega, Madison, WI). The −1,749/+132pΔ-6 desaturase.LUC construct was prepared by removing the *Sac*I fragment −1,749/−118 from −6,249/+279 and inserting it into the *Sac*I site of the −118/+132pΔ-6 desaturase.LUC construct. The reporter −6,249/+132pΔ-6 desaturase.LUC was prepared by linking the *Kpn*I-*Pst*I fragment of −6,249/−1,581 with the corresponding sites located in −1,749/+132pΔ-6 desaturase.LUC. Constructs −417/+132pΔ-6 desaturase.LUC and −283/+132pΔ-6 desaturase.LUC were generated by 5′-digestion of −1,749/+132pΔ-6 desaturase.LUC using exonuclease III and mung bean nuclease. Mutation of the DR-1 located at −385/−373 of the human Δ-6 desaturase gene was accomplished using the vector −417/+132pΔ-6 desaturase.LUC as the template in a polymerase chain reaction procedure that employed 5′-CCTCCGGTACCCGGGGCCGGAGAG TGGGGGAGtGAGGcGaTCGGACACG-3′ as the forward primer (mismatched bases indicated by lower-case letters). The polymerase chain reaction DNA product was then digested with *Kpn*I and *Nae*I, and ligated to the *Kpn*I and *Sma*I sites of the pGL 3 basic vector (i.e., −417mp Δ-6 desaturase.LUC). Sequence fidelity and the introduced mutations were verified by sequencing.

Site of transcription initiation

The start site of transcription for Δ-6 desaturase was mapped by a modification of the S1 nuclease method (26). A 5′-end labeled, single-strand 220 nucleotide (nt) DNA fragment corresponding to the 12–231 nts upstream of the translation start codon for human Δ-6 desaturase was synthesized using Klenow fragment. The labeled fragment was purified by electrophoresis in a 7% acrylamide gel. Total RNA (100 μg) extracted from HepG2 or glioma cells was mixed with 8 ng (15,000 dpm) of single-strand probe, and the mixture dried under vacuum. The pellet was resuspended in 25 μl of 80% formamide, 40 mM Hepes (pH 6.4), 1 mM EDTA, and 0.4 M NaCl, and incubated at 90°C for 5 min and then at 50°C overnight. The sample was subsequently digested with 800 units of S1 nuclease for 1 h at room temperature. The S1 nuclease-digested products were precipi-

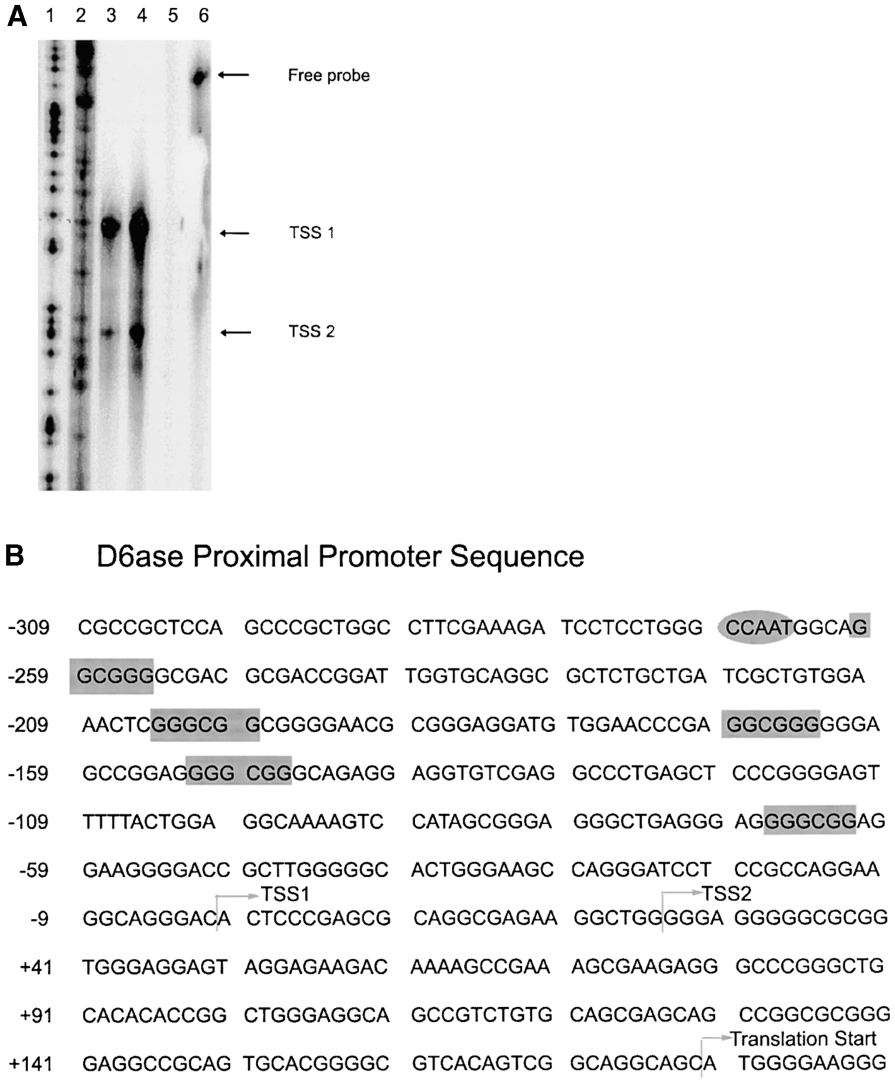

Fig. 4. Identification of the transcription initiation sites for the human Δ-6 desaturase gene. A: Transcription initiation sites for the human Δ-6 desaturase were identified using S1 nuclease analysis and 100 μg total RNA extracted from HepG2 cells or human glioma cells. Lanes 1 and 2 are adenine and thymidine DNA sequencing ladders, respectively. Lanes 3 and 4 depict the transcription initiation sites (TSS) for the Δ-6 desaturase gene in human glioma cells and the human hepatoma HepG2 cells, respectively. TSS 1 and TSS 2 are located 177 and 143 nucleotides (nts) upstream from the translation initiation codon, AUG. Lane 5 is the 220 nt probe subjected to S1 nuclease in the absence of RNA, and Lane 6 is the free radiolabeled 220 nt probe. B: Depicts the nt sequence for the −309/+141 region of human Δ-6 desaturase gene. The gray oval denotes a candidate consensus CCATT box, and the gray rectangles denote candidate Sp1 binding sites. The two major transcription start sites are marked as TSS 1 and TSS 2, and the translation start site for the transcript is noted at +180.

tated with ethanol. The precipitate was dried and resuspended in 1× Tris-EDTA (pH 7.5), boiled with formamide loading dye, and the resulting fragments were separated by electrophoresis in a 5% polyacrylamide, 7 M urea denaturing gel.

Cell culture and transfection

HepG2 cells and CV-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (11, 27, 28). The impact of 18:1(n-9) and 20:4(n-6) on endogenous HepG2 expression of Δ-6 desaturase was determined by quantifying Δ-6 desaturase mRNA abundance in confluent HepG2 cells that had been incubated for 36 h with 0 μM, 10 μM, 50 μM, or 200 μM albumin-bound (fatty acid-albumin ratio of 4:1, w/w) 18:1(n-9) or 20:4(n-6) (11). The influence of the PPARα-specific ligand activator, WY 14,643, on endogenous Δ-6 desaturase expression was determined by measuring the Δ-6 desaturase mRNA abundance in HepG2 cells that had been transfected with a PPARα expression vector (0.2 μg pSG5.mPPARα) and treated for 36 h with 100 μM WY 14,643 dissolved in dimethylsulfoxide or with vehicle alone. The influence of fatty acids on human Δ-6 desaturase promoter activity was determined by transfecting HepG2 cells in 6-well plates with 1.8 μg of −1,749, −668, −417, −417m, or −283pΔ-6 desaturase.LUC and incubating the cells for 36 h with 0.4% fatty acid-free BSA or 100 μM albumin-bound 18:1(n-9) or 20:4(n-6) (11). The effect of PPARα activation on human Δ-6 desaturase promoter activity was evaluated by

TABLE 2. Regulation of the human Δ-6 desaturase promoter by PUFAs and the peroxisome proliferator activated receptor α activator, WY 14,643

Construct	Treatments				
	None	18:1(n-9)	20:4(n-6)	−WY 14,643	+WY 14,643
	luciferase activity RLU/mg protein				
−1,749	141 ± 6	126 ± 7	69 ± 8[a]	162 ± 14	295 ± 10[b]
−668	ND	ND	ND	137 ± 22	283 ± 22[b]
−417	149 ± 8	124 ± 10	80 ± 8[a]	177 ± 19	377 ± 27[b]
−417m	138 ± 8	124 ± 8	76 ± 9[a]	155 ± 5	142 ± 7
−283	129 ± 11	127 ± 12	78 ± 13[a]	126 ± 6	116 ± 5
−118	ND	ND	ND	69 ± 11	56 ± 2

RLU, relative light unit. HepG2 cells were treated with 100 μM albumin-bound fatty acid or WY as described in Materials and Methods. Cells treated with (+) and without (−) WY were cotransfected with pSG5.m peroxisome proliferator-activated receptor (PPAR)α. Luciferase activity in cells treated with WY, but transfected with empty PPARα vector or not transfected with pSG5.mPPARα was 89% + 8% and 93% + 2% cells, respectively, of the luciferase activity observed in cells treated with vehicle alone. Values are expressed as means ± SEM for n = 2–4 separate experiments with 3–4 replicate plates in each experiment.

[a] 20:4(n-6) values are significantly ($P < 0.05$) lower than "no addition."

[b] Denotes significant ($P < 0.05$) effect of WY.

transfecting HepG2 cells in 6-well plates with 1.8 μg of −1,749, −668, −417, −417m, −283, or −118 pΔ-6 desaturase.LUC and treating the cells with 100 μM WY 14,643. The dependence of Δ-6 desaturase promoter activity on PPARα was further evaluated in CV-1 cells that had been cotransfected with 0.2 μg pSG5.mPPARα plus 0.2 μg pSG5.retinoic acid receptor X (RXR)α. The specificity of the PPARα effect was further evaluated by transfecting HepG2 and CV-1 cells with pSG5 vector lacking the open reading frame for PPARα, and by treating cells with WY 14,643 that had not been transfected with either pSG5 or pSG5.mPPAR, but had been treated with transfection reagent. The influence of hepatic nuclear factor 4 (HNF-4) on human Δ-6 desaturase promoter activity was examined by transfecting CV-1 cells with the expression vector pCMV.HNF-4. Transfection of HepG2 and CV-1 cells was conducted using cells seeded onto 6-well plates and grown to 65–75% confluence. At this point, the cells were transfected with the respective vector(s) by incubating them for 12 h in a serum-free transfection medium containing the lipofectamine (Life Technology, Rockville, MD) (28). After a 12 h incubation period, the transfection medium was removed and replaced with a serum-free medium containing 10^{-7} M insulin and dexamethasone, 10 μg/ml α-tocopherol plus the fatty acid, or WY 14,643. Cells were harvested after 36 h treatment using lysis buffer (Promega). Luciferase activity was quantified and is expressed as relative light units (RLUs) per μg protein (28). Transfection efficiency was evaluated by cotransfection with 0.2 μg/well pCMV.βgal and determining the activity of β-galactosidase.

Electrophoretic mobility shift assay

Nuclear proteins for use in electrophoretic mobility shift assays (EMSAs) were extracted from HepG2 cells transfected with pSG5.mPPARα, empty pSG5, or no vector, and treated with or without 100 μM WY 14,643 for 36 h (28, 29). Briefly, cells were washed twice in ice-cold PBS, scraped into 1.5 ml microfuge tubes, and centrifuged at 500 *g* for 20 s in a microcentrifuge. The cell pellet was then resuspended in 1 ml ice-cold buffer A [10 mM Hepes (pH 7.9), 1 mM EDTA, 10 μM KCl, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 μg/ml pepstain A, 10 μg/ml leupeptin, and 2 μg/ml aprotinin]. After incubation on ice for 20 min, Nonidet P-40 was added to a final concentration of 0.5%. After vigorous vortexing for 20 s, the cell suspension was centrifuged at 15,000 *g* for 30 s to collect the nuclei. The nuclei were resuspended in 10 vol of buffer B [10 mM Hepes (pH 7.9), 1 mM EDTA, 0.42 M NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 μg/ml pepstain A, 10 μg/ml leupeptin, and 2 μg/ml aprotinin], incubated on ice for 20 min, and centrifuged at 15,000 *g* for 10 min at 4°C. The resulting supernatants were stored at −80°C. In vitro translated mouse PPARα and rat RXRα were synthesized using the TNT-coupled reticulocyte lysate system (Promega). Double-strand oligonucleotides composed of the following sequences were used for EMSAs and competition analyses: human Δ-6 desaturase DR-1 (−385/−373), 5′GTGGGGGAGGGAGGAGGTCGGACACGG-3′; mutated Δ-6 desaturase DR-1, 5′-GTGGGGGAGtGAGGcGaTCGGACACGGTA-3′; rat AOX DR-1/PPAR-response element (RE), GGGGACCAGGACAAAGGTCAAGCAGCCAT. The DR-1/PPRE sequences are underlined, and mutated bases are shown in lowercase letters. Annealed oligonucleotides were end-labeled with [γ^{-32}p]ATP (Amersham) using T4 polynucleotide kinase. A 15 μl reaction containing 0.5–1.0 ng (50,000 cpm) of labeled DR-1/PPAR-RE and 4 μg of nuclear extract or in vitro translated 2 μl of PPARα and/or RXRα were incubated for 30 min on ice in a buffer containing 20 mM Hepes (pH 8.0), 60 mM KCl, 1 mM dithiothreitol, 10% glycerol, and 0.2 μg poly(dI-dC). In the super-shift analyses, 2 μg of H-98 anti-PPARα (Santa Cruz Biotechnology, Santa Cruz, CA) was incubated with nuclear protein extracts on ice for 12 h before labeled probe was added. After incubation, DNA-protein complexes were separated by electrophoresis on 5% polyacrylamide gel in Tris-glycine buffer at 4°C and visualized by autoradiography (28).

RESULTS

Transcriptional regulation of Δ-6 desaturase by PUFA and WY 14,643

Feeding rats or mice a high-carbohydrate, fat-free diet supplemented with n-6 and n-3 PUFA lowers the hepatic mRNA abundance and enzymatic activity of Δ-6 desaturase and Δ-5 desaturase (15, 16). On the other hand, feeding the PPARα-specific activator WY 14,643 leads to a marked increase in the mRNA abundance and enzymatic activity of Δ-6 desaturase and Δ-5 desaturase (19). Nuclear run-on assays revealed that the ingestion of safflower oil rich in 18:2(n-6) or dietary fish oil rich in 20- and 22-carbon n-3 fatty acids reduced the hepatic abundance of Δ-6 desaturase mRNA by inhibiting the rate of Δ-6 desaturase gene transcription 60% and greater than 95%, respectively (**Table 1**; **Figs. 1**, **2**). The extent of inhibition by dietary PUFA was comparable to that of the fatty acid synthase gene, a gene whose transcription is well recognized as being inhibited by dietary PUFA (9). The inhibition of Δ-6 desaturase gene expression was specific for n-6 and n-3 PUFA, because feeding comparable amounts of triolein [i.e., 18:1(n-9)] did not lower the rate of Δ-6 desaturase gene transcription or the hepatic abundance of Δ-6 desaturase and Δ-5 desaturase mRNA (Table 1; Figs. 1, 2). In contrast to the effects of dietary PUFA, ingestion of WY 14,643 increased the level of rat liver Δ-6 desaturase mRNA by inducing the rate of Δ-6 desaturase gene tran-

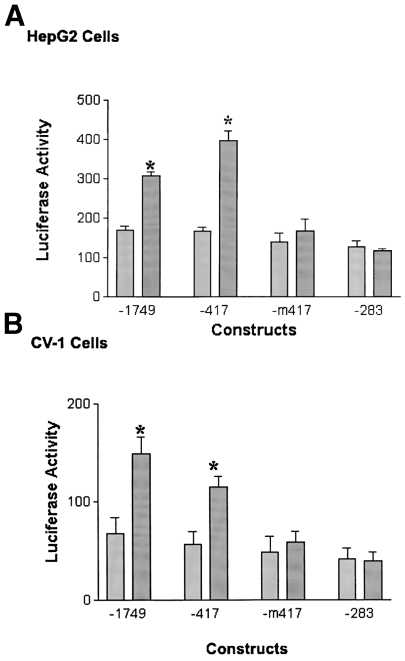

Fig. 5. The WY responsive sequence for the human Δ-6 desaturase gene resides between −385 and −373. HepG2 (A) and CV-1 (B) cells were transfected with the PPARα expression vector pSG5.mPPARα and cotransfected with Δ-6 desaturase luciferase reporter (pΔ-6 desaturase.LUC) constructs containing 5′-deletions, or mutations in the −385/−373 imperfect direct repeat-1 (DR-1) (−m417). Following transfection, cells were treated for 36 h with 100 μM WY (dark gray bar) or dimethylsulfoxide vehicle (light gray bar). Luciferase activity is expressed as relative light unit (RLU) per μg protein total cellular protein. Average luciferase activity in cells transfected with various pΔ-6 desaturase.LUC constructs, but not transfected with pSG5.mPPARα, averaged 122 ± 8 and 131 ± 10 RLU/μg for cells incubated with and without WY. Data represent means ± SE of n = 5 replicates per construct. Each point was replicated by 2–4 independent experiments. Asterisk indicates that WY significantly increased luciferase expression ($P < 0.05$).

scription more than 500% (Table 1; Figs. 1, 2). As expected, WY 14,643 increased the rate of gene transcription for the PPARα target gene AOX more than 15-fold (Table 1; Fig. 1). Unfortunately, the impact of dietary PUFA and WY 14,643 on the rate of Δ-5 desaturase gene transcription could not be determined because the hybridization signal was below the level of reliable detection. Expression of the human Δ-6 desaturase gene was also inhibited by PUFA and induced by PPARα activators (**Fig. 3**). Specifically, treating HepG2 cells with 20:4(n-6) resulted in a dose-dependent reduction in the cellular abundance of Δ-6 desaturase mRNA (Fig. 3), and supplementing the media with 100 μM WY 14,643 significantly increased the cellular abundance of Δ-6 desaturase in HepG2 cells that expressed PPARα (Fig. 3).

Identification of the start of transcription for the human Δ-6 desaturase

A 100 Kbp human Chr 11 fragment (Chr 11q12.2 PAC clone AC 004228) was determined to contain the entire transcribed sequences for the Δ-6 desaturase and Δ-5 desaturase genes, as well as containing the 11.2 Kbp intervening sequence lying between the two genes. S1 nuclease analysis revealed that transcription for the human Δ-6 desaturase gene was initiated at multiple sites in both human glioma and liver cells (**Fig. 4**). The two major points for transcription initiation were located at −177 and −143 nt from the ATG codon (Fig. 4A). The presence of multiple transcription initiation sites is consistent with the fact that the human Δ-6 desaturase gene does not appear to contain a classic TATA box (Fig. 4B). For the purpose of describing the location of *cis*-acting elements in the 5′-flanking sequence of the human Δ-6 desaturase gene, the −177 start point is considered +1. Although the human Δ-6 desaturase gene lacks a TATA-box, the G/C rich region between −280 and +1 contains several candidate binding sites for Sp1 (Fig. 4B). Moreover, a CCAAT-box motif is located at −269/−265, and the −289/−200 region contains recognition sequences for the enhancer factors, sterol regulatory element binding protein-1 (SREBP-1) and NF-Y (22).

Mapping the WY 14,643 response element in the human Δ-6 desaturase gene

PUFAs reportedly are activators of PPARs (30). Thus, we wanted to ascertain if the site in the human Δ-6 desaturase promoter responsible for the WY 14,643 induction of Δ-6 desaturase expression overlapped with the same region that conferred 20:4(n-6) inhibition. Treating HepG2 cells with 100 μM albumin-bound 20:4(n-6) reduced luciferase expression from −1,749/+132pΔ-6 desaturase.LUC 50% ($P < 0.05$), while incubating hepatocytes with the PPARα activator, WY 14,643, increased luciferase expression 2-fold ($P < 0.05$) (**Table 2**; **Fig. 5A**). Arachidonate's 20:4(n-6) inhibitory influence on human Δ-6 desaturase promoter activity was not mimicked by 18:1(n-9) (Table 2). Moreover, luciferase expression in HepG2 cells transfected with pSV40.LUC was not suppressed by 100 μM 20:4(n-6); i.e., luciferase activity (RLU/μg) was 60 ± 4 and 67 ± 4 in HepG2 cells treated with and without 20:4(n-6), respectively. The 20:4(n-6) suppression of the human Δ-6 desaturase promoter was not altered when the sequences between −1,749 and −417 were deleted (Table 2). Similarly, deleting the −1,749/−417 region did not reduce the WY 14,643 induction of Δ-6 desaturase promoter (Table 2; Fig. 5). Stimulation of human Δ-6 desaturase promoter activity by WY 14,643 required the coexpression of PPARα. Luciferase activity in HepG2 cells transfected with −417/+132pΔ-6 desaturase.LUC but not cotransfected with pSG5.mPPARα was 139 ± 17 and 138 ± 13 (RLU/μg protein) in the absence and presence of WY 14,643, respectively, and luciferase activity in HepG2 cells transfected with empty pSG5 was 144 ± 15 and 134 ± 11 in the absence and presence of WY 14,643, respectively. Deletion of the region between −417 and −283 eliminated the WY 14,643 enhancement of the Δ-6 desaturase promoter, but the deletion had no effect on the 20:4(n-6) inhibition of luciferase expression (Table 2; Fig. 5). Sequence analysis revealed that the region between −417 and −283 contained an imperfect DR-1 ($_{-385}$AGGGAG g

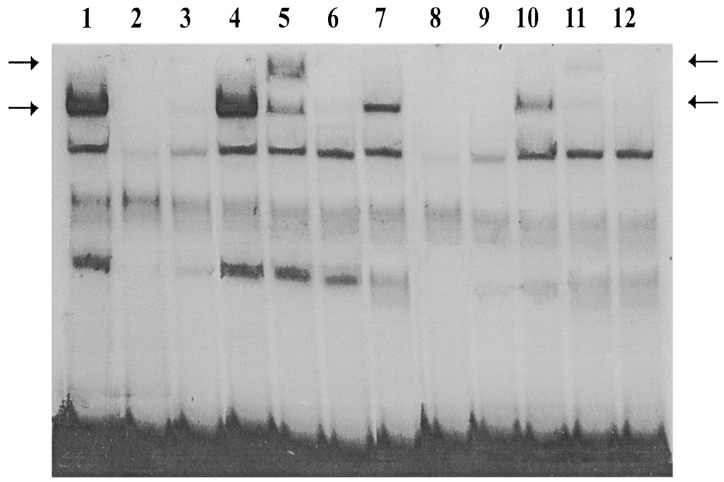

Fig. 6. The −385/−373 DR-1 of the human Δ-6 desaturase gene binds PPARα and retinoic acid receptor X (RXR)α heterodimers from HepG2 cells. Electrophoretic mobility shift assays (EMSAs) were conducted with radiolabeled DR-1/PPAR-RE of rat AOX (DR-1, Lanes 1 through 6) or −385/−373 DR-1 of human Δ-6 desaturase (DR-1, Lanes 7 through 12). Nuclear protein extracts prepared from HepG2 cells expressing mouse PPARα (pSG5.mPPARα) (see Materials and Methods) were employed in the EMSAs of Lanes 1 through 5 (AOX DR-1), and Lanes 7 through 11 (Δ-6 desaturase DR-1). The electromobility shift patterns acquired using nuclear protein extracts from HepG2 cells not transfected with pSG5.mPPARα are depicted in Lane 6 (AOX DR-1) and Lane 12 (Δ-6 desaturase DR-1). Competition experiments were conducted with 200-fold molar excess of unlabeled rat AOX DR-1/PPAR-RE (Lanes 2 and 9), human Δ-6 desaturase DR-1 (Lanes 3 and 8), and mutated Δ-6 desaturase DR-1 (Lanes 4 and 10). The lower arrow indicates the position of the PPARα-RXRα heterodimer-DNA complex, and the upper arrow indicates the location of the super-shifted complexes resulting from treatment with anti-PPARα (Lanes 5 and 11).

AGGTCG$_{-373}$) that was a candidate PPAR-RE. The introduction of three nt mutations into the candidate DR-1 sequence (i.e., 5′-AGG/tGAG g A/cGG/aTCG-3′) eliminated >90% of the WY 14,643 stimulation of Δ-6 desaturase promoter activity in both HepG2 and CV-1 cells (Table 2; Fig. 5, −417mpΔ-6 desaturase.LUC). On the other hand, luciferase expression in HepG2 cells transfected with −417mpΔ-6 desaturase.LUC continued to be suppressed by 20:4(n-6) (Table 2). These data indicate that the PPARα and PUFA response sequences responsible for governing the human Δ-6 desaturase gene were located in different regions of the Δ-6 desaturase gene and functioned independently of each other.

A DR-1 also binds HNF-4 (31). However, expressing HNF-4 in CV-1 cells had no effect on Δ-6 desaturase promoter activity (i.e., luciferase expression from −1,749/+132pΔ-6 desaturase.LUC, −417/+132pΔ-6 desaturase.LUC, and −283/+132pΔ-6 desaturase.LUC was 47 ± 13, 42 ± 17, and 48 ± 16 RLU/μg protein in CV-1 cells cotransfected with an HNF-4 expression vector, respectively, and 41 ± 9 RLU/μg protein in CV-1 cells transfected with empty HNF-4 vector). In contrast, luciferase expression in CV-1 cells transfected with −7,382/−6,970/−250pFAS.LUC, a construct that contains an HNF-4 response element (RE) from the rat fatty acid synthase promoter, was stimulated 3-fold (data not shown).

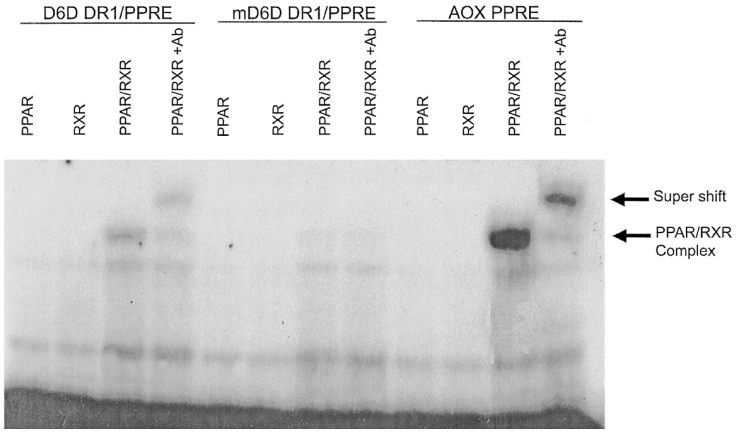

Fig. 7. The −385/−373 DR-1 from the human Δ-6 desaturase gene binds PPARα and RXRα heterodimers. EMSAs were conducted with in vitro-translated mouse PPARα and/or rat RXRα plus radiolabeled −385/−373 Δ-6 desaturase DR-1 (Δ-6 desaturase DR-1), mutated −385/−373 DR-1 (mtΔ-6 desaturase), or the DR-1/PPAR-RE of rat AOX imperfect DR-1 (see Materials and Methods). The lower arrow indicates the position of the PPARα-RXRα heterodimer-DNA complex, and the upper arrow indicates the location of the super-shifted complexes resulting from treatment with anti-RXRα (PPAR/RXR+AB).

PPARα binding to the DR-1 of the human Δ-6 desaturase gene

Nuclear proteins extracted from HepG2 cells expressing PPARα readily interacted with the DR-1 of the human Δ-6 desaturase gene and the DR-1/PPAR-RE of the rat peroxisomal OAX gene (**Fig. 6**), but nuclear protein extracts from HepG2 cells not transfected with the PPARα expression vector displayed little or no binding to either the Δ-6 desaturase or the AOX DR-1. The uppermost band in the EMSA was most prevalent in HepG2 cells transfected with pSG5.mPPARα (Fig. 6, Lanes 1 and 7), and it was the only band super-shifted by treatment with anti-PPARα (Fig. 6, Lanes 5 and 11) or by anti-RXRα (data not shown). Unlabeled DR-1 from the AOX and Δ-6 desaturase genes competed for protein binding with the labeled DR-1 of the respective genes (Fig. 6, Lanes 2, 3, 8, and 9). Mutating the DR-1 of the Δ-6 desaturase gene prevented the sequence from competing for protein interactions with either the DR-1/PPAR-RE of AOX or the DR-1 of Δ-6 desaturase (Fig. 6, Lanes 4 and 10). The DR-1 from the human Δ-6 desaturase gene and the DR-1/PPAR-RE of the rat AOX gene were also found to specifically interact with a mixture of in vitro-translated PPARα and RXRα, but by themselves, neither PPARα nor RXRα were able to bind to the respective DR-1 sequences (**Fig. 7**). Mutation of the Δ-6 desaturase DR-1 (i.e., 5′-GG/tGAG g A/cGG/aTCG-3′) completely eliminated PPARα-RXRα binding (Fig. 7), and treatment of the PPARα-RXRα-DR-1 complex with anti-RXRα super-shifted both the Δ-6 desaturase DR-1 and the classical DR-1/PPAR-RE of the rat AOX gene (Fig. 7). Collectively, these data indicate that the imperfect DR-1 located at −385/−373 of the human Δ-6 desaturase gene possesses the ability to bind the heterodimer PPARα-RXRα and subsequently to function as a PPAR-RE.

DISCUSSION

Twenty- and 22-carbon n-6 and n-3 fatty acids are essential for neural development, eye function, reproduction, and cellular differentiation (1–10). The hepatic synthesis of 20:4(n-6) and 20:5(n-3) from 18:2(n-6) and 18:3(n-3) involves Δ-6 desaturase and Δ-5 desaturase, the associated elongases. Flux through the hepatic Δ-6 desaturase pathway appears to be dictated by Δ-6 desaturase enzymatic activity, and this in turn is determined by the hepatic abundance of Δ-6 desaturase mRNA (15–22). While consumption of either n-6 or n-3 fatty acid substrates or products for Δ-6 desaturase will lower Δ-6 desaturase and Δ-5 desaturase mRNA abundance and hence flux through the pathway, we have recently determined that the bioactive inhibitory lipid is not the 18:2(n-6) or 18:3(n-3) substrate, but rather the inhibitor is a polyenoic fatty acid metabolite of the Δ-6 desaturase pathway (32).

In this report, we demonstrate that PUFAs lower the hepatic abundance of Δ-6 desaturase mRNA by inhibiting the rate of Δ-6 desaturase gene transcription. This inhibition of Δ-6 desaturase gene transcription applies to both the rat and human Δ-6 desaturase genes (Tables 1, 2). Transfection reporter assays with HepG2 cells revealed that the PUFA response sequences for the human Δ-6 desaturase gene resided within the proximal promoter region of −283/+1 (Table 2). Recently, Nara et al. (22) reported that the E-box-like sterol RE located at −222/−231 and the NF-Y recognition site at −273/−268 are both required for PUFA suppression of the human Δ-6 desaturase promoter. SREBP-1 and NF-Y have been implicated in the PUFA inhibition of transcription of other lipogenic genes, including FAS and stearoyl-CoA desaturase-1 (33, 34). Dietary PUFAs exert their inhibitory influence by lowering the nuclear content of mature SREBP-1 protein and by interfering with the transactivation action of NF-Y (11, 33–37). PUFAs decrease the nuclear content of SREBP-1 in two ways. First, they inhibit the proteolytic release of mature SREBP-1 from its membrane-anchored precursor (35, 37). Second, PUFAs accelerate the decay of SREBP-1 mRNA and consequently lower the abundance of SREBP-1 mRNA, which in turn leads to a reduction in the amount of membrane-anchored precursor SREBP-1 protein (36). The mechanism by which PUFAs interfere with NF-Y action is unclear, but it may involve a posttranslational modification of NF-Y (34).

While dietary PUFA suppressed Δ-6 desaturase and Δ-5 desaturase gene expression, ingestion of the PPARα activator WY 14,643 increased hepatic expression of Δ-6 desaturase and Δ-5 desaturase (Table 1; Figs. 1, 2). The PPARα induction of Δ-6 desaturase and Δ-5 desaturase gene expression is accompanied by an enhanced hepatic production of 20- and 22-carbon n-6 and n-3 PUFA (21), and by an increased level of 20- and 22-carbon PUFA in peripheral tissues (H. Y. Cho and S. D. Clarke, unpublished observations). This PPARα-dependent increase in synthesis of 20- and 22-carbon PUFA may explain how PPARα activators (e.g., WY 14,643) indirectly lowered the nuclear content of hepatic SREBP-1, and consequently reduced the transcription of lipogenic genes such as fatty acid synthase, which lack a functional PPARα RE [Table 1; Fig. 1, and (11)].

PPARα modulates the transcription of a gene by interacting with its heterodimer partner RXRα and subsequently binding to a hexameric (AGGTCA) DR with a single nt spacer. The 5′-flanking sequence of the human Δ-6 desaturase gene was found to contain an imperfect DR-1 ($_{-385}$AGGGAGgAGGTCT$_{-373}$). Binding of PPARα to the −385/−373 Δ-6 desaturase DR-1 required RXRα (Fig. 7), and transfection reporter analyses demonstrated that the DR-1 imparted PPARα responsiveness to the human Δ-6 desaturase promoter, i.e., expression of PPARα in HepG2 and CV-1 cells significantly enhanced human Δ-6 desaturase promoter activity in response to WY 14,643 (Fig. 5). Nevertheless, the physiological role of the DR-1 in human Δ-6 desaturase gene transcription remains unclear because human tissues contain low amounts of PPARα (27), and because the imperfect DR-1 may be a possible recognition sequence for several tran-

scription factors including HNF-4, PPARγ, PPARδ, farnesoid X receptor, and chicken ovalbumin upstream promoter transcription factor (30, 38–40). In this regard, it is noteworthy that expression of HNF-4 in CV-1 cells did not alter Δ-6 desaturase promoter activity, but this does not eliminate the possibility that transcription factors other than HNF-4 or PPARα may recognize the DR-1 site from the human Δ-6 desaturase gene. Nevertheless, our data strongly suggest that the −385/−373 DR-1 of the human Δ-6 desaturase is a functional response element that plays a role in the expression of Δ-6 desaturase and ultimately the synthesis of 20- and 22-carbon PUFA.

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